

Refine Search

Search Results -

Terms	Documents
L2 and destabiliz\$	2

Database:

US Pre-Grant Publication Full-Text Database
 US Patents Full-Text Database
 US OCR Full-Text Database
 EPO Abstracts Database
 JPO Abstracts Database
 Derwent World Patents Index
 IBM Technical Disclosure Bulletins

Search:

L4

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Recall Text

Clear

Interrupt

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 DATE: Tuesday, August 01, 2006 [Printable Copy](#) [Create Case](#)

<u>Set</u> <u>Name</u> side by side	<u>Query</u>	<u>Hit</u> <u>Count</u>	<u>Set</u> <u>Name</u> result set
<i>DB=USPT,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR</i>			
<u>L4</u>	L2 and destabiliz\$	2	<u>L4</u>
<u>L3</u>	(liposome or vesicle) adj10 (cholate or brij\$ or myrj\$) same destabiliz\$	0	<u>L3</u>
<u>L2</u>	(liposome or vesicle) adj10 (cholate or brij\$ or myrj\$)	38	<u>L2</u>
<u>L1</u>	(liposome or vesicle) adj5 cholate	14	<u>L1</u>

END OF SEARCH HISTORY

[First Hit](#) [Fwd Refs](#)[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)

Generate Collection

Print

L1: Entry 5 of 14

File: USPT

Feb 15, 2000

DOCUMENT-IDENTIFIER: US 6025333 A

TITLE: Treatment of CNS tumors with metalloprotease inhibitors

Detailed Description Text (166):

Liposomes were prepared in medium A by the cholate method (Brunner, et al., 1978, J. Biol. Chem. 253:7538-7546). Protein was solubilized in 2% SDS (in medium A plus protease inhibitors); insoluble protein was sedimented and discarded. Solubilized protein was precipitated with a 30-fold excess of acetone. To obtain reproducible yields, acetone precipitation was allowed to proceed for 15 hours at 4.degree. C. Protein extracts from tissues were prepared by homogenization of minced tissue with a glass-teflon potter in 2% SDS-containing, protease inhibitors-supplemented, medium A. Solubilized protein was then precipitated with ice-cold acetone as described above. Extracts from cultured cells were prepared by, first, detaching the cells with a rubber policeman in the presence of PBS plus EDTA plus protease inhibitors, and by then homogenizing suspended cells with a glass-teflon potter. Upon low-speed pelleting of nuclear material, 2% SDS was added to supernatants and solubilized protein was precipitated with ice-cold acetone. In all cases, acetone-precipitated protein was sedimented (10,000.times. g, 15 minutes) and resuspended at 1 mg/ml in medium A with 2.5% cholate. Phospholipids (phosphatidylcholine/phosphatidylserine, 10:1) dissolved in medium A with 2.5% cholate were then added (.about.5-10:1 ratio of added phospholipid to protein) and liposomes were formed on a Sephadex G-50 column. When gel-extracted protein was reconstituted, precipitated protein was resuspended at .about.50 .mu.g/ml and phospholipid to protein ratios were up to 100:1.

[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)

[First Hit](#) [Fwd Refs](#)[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)[Generate Collection](#)[Print](#)

L1: Entry 6 of 14

File: USPT

Jan 26, 1999

DOCUMENT-IDENTIFIER: US 5863556 A

**** See image for Certificate of Correction ****

TITLE: Preparations for the external application of antiseptic agents and/or agents promoting the healing of wounds

Brief Summary Text (65):

The liposomes are generally formed by agitating said aqueous system in the presence of said film formed by the lipid components. At this stage, further additives can be added to improve liposome formation; for example, sodium cholate can be added. Liposome formation can also be influenced by mechanical action such as pressure filtration through, for example, polycarbonate membranes, or centrifuging. Generally, the raw liposome dispersion will be washed, for example, with electrolyte solution as used in preparing the above-described solution of the active agent.

[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)

[First Hit](#) [Fwd Refs](#)[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)[Generate Collection](#)[Print](#)

L1: Entry 7 of 14

File: USPT

Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5853753 A

**** See image for Certificate of Correction ****

TITLE: Liposomes, method of preparing the same and use thereof in the preparation of drugs

Brief Summary Text (144):

As already described in sections I and II, the pain caused by viral infection or allergy disappears immediately in most cases after a liposome application. At least part of the mediators which are released by virally caused tissue lesions or allergic reactions are algogenic substances which alarm the nociceptors of the corresponding tissue. An objective assessment which furnishes proof of an action of physiological liposomes on nerve activity follows from the finding determined in Example 3 on the isolated intestine of a guinea pig. The pronounced inhibition of nerve activity in the case of relatively low liposome concentrations furnishes proof of the surprising efficacy of the cholate liposomes. The cholate-free liposomes used as a control lead to a stimulation of the nerves. Since an influence of the liposomes on the ion composition of the fluid is negligibly small due to the relatively small amounts of liposomes in the organ bath, but since cholate-free liposomes effect such a pronounced stimulation, it must be assumed that these liposomes, too, directly interact with the tissue or the cells. It is known from numerous experiments that surfactant-containing liposomes have a greater loss of enclosed hydrophilic small ions or molecules than surfactant-free liposomes. The cholate liposomes which are here used also have this property; i.e. surfactant-containing phospholipid membranes have a slightly increased permeability (in both directions) for such substances. When liposomes having a relatively great cholate amount (as the ones used here) fuse with cells, the entrained surfactants transmit the slightly increased membrane permeability, for instance for ions such as Na.sup.+ and K.sup.+, to the cell membranes. The membrane potential (resting potential) is shifted on account of the now increased ion flow towards the "firing level" (10). Although this increases the excitability of the cell, the decrease in action potential corresponding to the depolarization simultaneously decreases the transmitter substances to be released (less vesicles are released), thereby effecting a reduced excitation of the postsynaptic cell (4). This presynaptic efficiency could be confirmed in further experiments with acetyl choline- or histamine-stimulated isolated guinea pig intestine. By contrast, a change in the membrane potential away from the "firing level" follows for a fusion of surfactant-free liposomes with cells, which consequently entails an increased action potential and an increased release (increased vesicle release) of transmitter substance. This leads to the observed stimulation of the intestinal contraction.

Brief Summary Text (197):

If the interaction of liposomes and cells corresponds to a fusion, the following events might take place: After fusion of the liposome membrane with the cell membrane, a membrane piece having a size of about 0.1 μm^2 and increased permeability is incorporated through a liposome having a diameter of about 200 nm into the cell membrane (500 m^2 outer membrane surface at a cell diameter of 12.6 μm). As a result of this "new membrane piece" having a size of 0.1 μm^2 , there is an increase in the inflow and outflow of ions. However, since lipids, and presumably above all surfactants, diffuse laterally within the lipid double membrane at a rate of 2 $\mu\text{m}/\text{sec}$ (7), the liposomal membrane piece

already starts to flow apart at the time of the fusion, i.e., liposomal and cellular membrane components are mixed, which rapidly leads to a decrease in the ion permeability at this place of the cell membrane. A propagation of the liposomal membrane components to three to four times the area (0.3 to $0.4 \mu\text{m}^2$) might already lead to a substantial decline in the permeability (derived from experiments with liposomes having a lower cholate proportion). The high rate of lateral lipid diffusion of $2 \mu\text{m}/\text{sec}$ might therefore "seal" the originally $0.1 \mu\text{m}^2$ - sized liposomal membrane piece in fractions of a second for the greatest part. It is not until an increased number of liposomes have fused with a cell that the surfactants introduced into the cell membrane lead to a change in permeability regarding the whole cell membrane. It can be shown in vitro that cells react negatively to a very short-time (15-30 min) offer of very high liposome concentrations. After a few hours, however, the cells will fully recover therefrom. This regeneration can, inter alia, be explained by the fact that 50% of the plasma membrane are internalized per hour (depending on the cell type) and that consequently half of the cell membrane is replaced every hour by membrane material from the interior of the cell. The proportion of the cell membrane in the total membrane material of a cell amounts to a few percent only (in a liver cell to about 2%). The original permeability of the cell membrane is therefore re-established within a relatively short period of time.

[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)

[First Hit](#) [Fwd Refs](#)[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)[Generate Collection](#)[Print](#)

L4: Entry 1 of 2

File: USPT

Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5853753 A

**** See image for Certificate of Correction ****

TITLE: Liposomes, method of preparing the same and use thereof in the preparation of drugs

Brief Summary Text (17):

Every bile acid derivative can be used according to the invention. Preferred bile acid derivatives are sodium cholate, sodium deoxycholate, sodium glycocholate, sodium taurocholate, sodium taurodeoxycholate, sodium ursocholate, and sodium chenoxocholate. The added bile acid and/or the derivatives thereof lead to a decrease in the surface tension of the liposomes and thus to an increased fusogenity which facilitates the fusion of the liposomes with body cells. It is also assumed that a presumably slight change in membrane potential which is more or less limited in time takes place on account of an interaction between surface tension and membrane potential after fusion of the liposomes with cells. For instance, Olson et al. (10) write that a weak depolarization of the transmembrane potential will take place a few minutes after rat liver cells have been treated with taurocholate. Furthermore, it might be that the membrane components, depending on their spatial arrangement, are subjected to certain spatial changes by the bile acid or the derivatives thereof, the changes being expressed in a changed permeability of the membrane or a changed production rate for "second messenger". The surfactant contained in the liposome must, however, not lead to a destabilization of either the liposome membrane, or, after fusion, of the cell membrane. Among all of the tested surfactants, bile acid and the derivatives thereof fulfill these requirements best. Moreover, it has been observed that the liposomes prepared in this way have been very stable for long periods of time.

Brief Summary Text (37):

The liposomes according to the invention are preferably used for preparing drugs. Surprisingly enough, it has been found that the liposomes of the invention are effective against a group of very different diseases, such as allergies, viral infections, inflammations, and a specific kind of pain. All of these diseases, however, have in common that they originate from a specific "lability" or "acceptance" of the plasma membrane of the affected cells. For instance, specific allergic reactions are triggered by the destabilization of the mast cell membrane, nerve impulses are generated by destabilized cell membranes, and viruses can only penetrate into those cells whose cell membrane exhibits the necessary acceptance at the time of infection (details will be described in the following sections I to V).

Brief Summary Text (73):

7. Liposomes can interact with viruses, possibly fuse with viruses and finally neutralize them. To this end, and in accordance with the results of Example 1, a certain number of liposomes are needed for forcing viruses to perform such an interaction. The effective density follows mainly from the size of the viruses and the liposomes. The neutralization itself could be performed, in the case of enveloped viruses, simply by exceeding a limit size of importance to the respective virus (each virus has a specific maximum size) and, in the case of naked viruses, by enveloping the same with the liposome membrane. It is however likely that the viruses are destabilized by the surfactants transmitted after fusion with the

liposomes.

Brief Summary Text (144):

As already described in sections I and II, the pain caused by viral infection or allergy disappears immediately in most cases after a liposome application. At least part of the mediators which are released by virally caused tissue lesions or allergic reactions are algogenic substances which alarm the nociceptors of the corresponding tissue. An objective assessment which furnishes proof of an action of physiological liposomes on nerve activity follows from the finding determined in Example 3 on the isolated intestine of a guinea pig. The pronounced inhibition of nerve activity in the case of relatively low liposome concentrations furnishes proof of the surprising efficacy of the cholate liposomes. The cholate-free liposomes used as a control lead to a stimulation of the nerves. Since an influence of the liposomes on the ion composition of the fluid is negligibly small due to the relatively small amounts of liposomes in the organ bath, but since cholate-free liposomes effect such a pronounced stimulation, it must be assumed that these liposomes, too, directly interact with the tissue or the cells. It is known from numerous experiments that surfactant-containing liposomes have a greater loss of enclosed hydrophilic small ions or molecules than surfactant-free liposomes. The cholate liposomes which are here used also have this property; i.e. surfactant-containing phospholipid membranes have a slightly increased permeability (in both directions) for such substances. When liposomes having a relatively great cholate amount (as the ones used here) fuse with cells, the entrained surfactants transmit the slightly increased membrane permeability, for instance for ions such as Na.sup.+ and K.sup.+, to the cell membranes. The membrane potential (resting potential) is shifted on account of the now increased ion flow towards the "firing level" (10). Although this increases the excitability of the cell, the decrease in action potential corresponding to the depolarization simultaneously decreases the transmitter substances to be released (less vesicles are released), thereby effecting a reduced excitation of the postsynaptic cell (4). This presynaptic efficiency could be confirmed in further experiments with acetyl choline- or histamine-stimulated isolated guinea pig intestine. By contrast, a change in the membrane potential away from the "firing level" follows for a fusion of surfactant-free liposomes with cells, which consequently entails an increased action potential and an increased release (increased vesicle release) of transmitter substance. This leads to the observed stimulation of the intestinal contraction.

Brief Summary Text (165):

Since bile acids have an antibacterial effect, the cholate liposomes of the invention also exhibit bactericidal effects, at least against cholate-sensitive bacteria; i.e., some infections of bacterial etiology can also be treated in the case of the above-mentioned diseases.

Brief Summary Text (197):

If the interaction of liposomes and cells corresponds to a fusion, the following events might take place: After fusion of the liposome membrane with the cell membrane, a membrane piece having a size of about $0.1 \mu\text{m}^2$ and increased permeability is incorporated through a liposome having a diameter of about 200 nm into the cell membrane (500 μm^2 outer membrane surface at a cell diameter of 12.6 μm). As a result of this "new membrane piece" having a size of $0.1 \mu\text{m}^2$, there is an increase in the inflow and outflow of ions. However, since lipids, and presumably above all surfactants, diffuse laterally within the lipid double membrane at a rate of 2 $\mu\text{m}/\text{sec}$ (7), the liposomal membrane piece already starts to flow apart at the time of the fusion, i.e., liposomal and cellular membrane components are mixed, which rapidly leads to a decrease in the ion permeability at this place of the cell membrane. A propagation of the liposomal membrane components to three to four times the area (0.3 to $0.4 \mu\text{m}^2$) might already lead to a substantial decline in the permeability (derived from experiments with liposomes having a lower cholate proportion). The high rate of lateral lipid diffusion of 2 $\mu\text{m}/\text{sec}$ might therefore "seal" the originally $0.1 \mu\text{m}^2$ -

sized liposomal membrane piece in fractions of a second for the greatest part. It is not until an increased number of liposomes have fused with a cell that the surfactants introduced into the cell membrane lead to a change in permeability regarding the whole cell membrane. It can be shown in vitro that cells react negatively to a very short-time (15-30 min) offer of very high liposome concentrations. After a few hours, however, the cells will fully recover therefrom. This regeneration can, inter alia, be explained by the fact that 50% of the plasma membrane are internalized per hour (depending on the cell type) and that consequently half of the cell membrane is replaced every hour by membrane material from the interior of the cell. The proportion of the cell membrane in the total membrane material of a cell amounts to a few percent only (in a liver cell to about 2%). The original permeability of the cell membrane is therefore re-established within a relatively short period of time.

[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)

[First Hit](#) [Fwd Refs](#)[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)**End of Result Set**

Generate Collection

Print

L4: Entry 2 of 2

File: USPT

Oct 31, 1995

DOCUMENT-IDENTIFIER: US 5462859 A

TITLE: Method of diagnosing irregularities in bile salt absorption via brush border membrane proteins

Detailed Description Text (5):

The mechanism of solubilization of brush border membrane vesicles has been proposed to involve the incorporation of bile salt into the lipid bilayer of the brush border membrane and the simultaneous flow of brush border membrane components to the micellar phase. In excess bile salt micelles, these two processes lead to the destabilization of the brush border membrane with the formation of mixed bile salt micelles containing both lipids and proteins of the brush border membrane.

Detailed Description Text (27):

(I) Isolated enterocytes closely resembled brush border membrane vesicles regarding the binding of sodium cholate. In all other properties tested so far, isolated enterocytes paralleled brush border membrane vesicles. These include the fast, protein-mediated uptake of cholesterol from bile salt micelles.

Detailed Description Text (78):

In this phase diagram the boundary between the lamellar and the micellar phase is practically identical with the line characteristic of a bile salt/egg PC mole ratio of 2. At this composition, the phase transition lamellar-to-micellar phase sets in, and at higher bile salt concentrations mixed micelles are present (Shankland, W. 1970. Chem. Phys. Lipids 4, 109-130). for instance, multilamellar egg PC vesicles of 10 mg lipid/ml (=13.3 mM) are solubilized by a total sodium cholate concentration of 30 mM (Brunner, J., Hauser, H., and Semenza, G. 1978. J. Biol. Chem. 253, 7538-7546).

[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)

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[First Hit](#)[Clear](#)[Generate Collection](#)[Print](#)[Fwd Refs](#)[Bkwd Refs](#)[Generate OACS](#)**Search Results - Record(s) 1 through 2 of 2 returned.**☐ 1. Document ID: US 5853753 A

L4: Entry 1 of 2

File: USPT

Dec 29, 1998

US-PAT-NO: 5853753

DOCUMENT-IDENTIFIER: US 5853753 A

**** See image for Certificate of Correction ****

TITLE: Liposomes, method of preparing the same and use thereof in the preparation of drugs

DATE-ISSUED: December 29, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maierhofer; Gunther	Munich			DE
Hofer; Paul	Dietersheim			DE
Rottmann; Oswald	Freising			DE

US-CL-CURRENT: 424/450

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	RMK	Drawings
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☐ 2. Document ID: US 5462859 A

L4: Entry 2 of 2

File: USPT

Oct 31, 1995

US-PAT-NO: 5462859

DOCUMENT-IDENTIFIER: US 5462859 A

TITLE: Method of diagnosing irregularities in bile salt absorption via brush border membrane proteins

DATE-ISSUED: October 31, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hauser; Helmut	CH-8092 Zurich			CH

US-CL-CURRENT: 435/29; 436/501, 436/504, 436/63, 436/811

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	RMK	Drawings
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Terms			Documents		
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Display Format:

[Previous Page](#)

[Next Page](#)

[Go to Doc#](#)